

Effect of Broiler Age, Feed Withdrawal, and Transportation on Levels of Coliforms, *Campylobacter*, *Escherichia coli* and *Salmonella* on Carcasses Before and After Immersion Chilling

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ABSTRACT A study was conducted to determine effects of bird age at slaughter, feed withdrawal, and transportation on levels of coliforms, *Campylobacter*, *Escherichia coli*, and *Salmonella* on carcasses before and after immersion chilling. Broilers were processed at 42, 49, and 56 d of age after a 12-h feed withdrawal period or a 0-h feed withdrawal period (full fed). At each age, broilers were processed from two commercial farms previously identified as *Campylobacter* positive. One week before slaughter, broilers were gavaged with nalidixic acid-resistant *Salmonella*. During bleeding, cotton plugs were inserted into the cloaca of each carcass. Whole-carcass rinses (WCR) were performed before and after immersion chilling with 20 ppm sodium hypochlorite, and rinses were analyzed for coliforms, *Campylobacter*, *E. coli* and *Salmonella*. Log₁₀

counts for coliforms, *Campylobacter*, and *E. coli* were ($P < 0.05$) affected by bird age at slaughter. Feed withdrawal (FW) affected only *Campylobacter* on carcasses of older broilers (56 d of age). Chilling with sodium hypochlorite resulted in log₁₀ reductions of 1.2, 1.3, 1.4, and 0.5 for coliforms, *Campylobacter*, *E. coli*, and *Salmonella*, respectively. Under the conditions of this experiment, it appears that contamination on the exterior of birds entering the processing facility is critical to carcass bacterial counts. Moreover, carcass bacterial counts did not vary when microbial counts of broilers were comparable. FW may increase prechill carcass counts for *E. coli* and *Campylobacter*, but it appears to have no effect on postchill carcass counts when sodium hypochlorite is used in the chilling operation.

(Key words: broiler transportation, *Campylobacter*, *Escherichia coli*, feed withdrawal, *Salmonella*)

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INTRODUCTION

According to the Centers for Disease Control and Prevention, there are approximately 2.4 million cases of human campylobacteriosis and 1.2 million cases of human salmonellosis infections in the U.S. each year (Centers for Disease Control, 2000, 2001). The most commonly implicated source for these illnesses is handling of raw poultry or consumption of undercooked poultry and poultry products (Smitherman et al., 1984; Altekruze, 1998; Centers for Disease Control and Prevention, 2000, 2001). Although the cost associated with campylobacteriosis has not been reported, the cost for salmonellosis has been estimated at more than \$1 billion annually in medical expenses and lost wages in the U.S. (Centers for Disease Control, 2001). Hence, any opportunity to directly intervene in the transmission of *Salmonella* and *Campylobacter* to poultry (birds and ul-

imately products) could have a significant impact on the U.S. economy.

In 1996, the USDA attempted to improve safety of poultry and meat by mandating the "Pathogen Reduction, Hazard Analysis and Critical Control Point (HACCP) System Final Ruling" (USDA, 1996). According to this ruling, establishments that slaughter poultry must meet performance standards for *Salmonella* and *E. coli* to remain in operation. Currently, poultry processing plants do not have a performance standard for *Campylobacter*; however, establishing a performance standard for this microorganism is being considered (USDA, 2000).

Numerous studies have focused on *Campylobacter* contamination of broilers prior to processing (Smitherman et al., 1984; Pokamunski et al., 1986; Hoop and Ehram, 1987; Jacobs-Reitsma et al., 1994; Stern et al., 1995; Slader et al., 2002). It has been reported that broilers may harbor *Campylobacter* in their intestinal tracts and *Campylobacter* may contaminate feed, litter, and drinking water during production (Smitherman et al., 1984; Genigeorgis et al., 1986; Jacobs-

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Abbreviation Key: FW = feed withdrawal; WCR = whole carcass rinse.

Reitsma et al., 1994). Without proper farm biosecurity, *Campylobacter* may be carried from house to house, or even farm to farm by workers and equipment (Slader et al., 2002). Another source of potential contamination is the transport coops used to deliver birds to the processing plant. Bolder (1998) reported that excretion of pathogenic bacteria during transportation to the processing plant would cause cross contamination among birds in transport coops. Altekruze (1998) reported that transportation of broilers to the processing plant could cause bacterial counts to increase up to 1,000-fold. Rigby et al. (1982) isolated *Salmonella* from 99% of the transport coops evaluated (98/99 coops) before loading with broilers. These same authors attribute *Salmonella* contamination of a previously negative flock to the transport coops (Rigby et al., 1982).

Stern et al. (1995) compared bacterial counts of carcasses from broilers slaughtered on the farm to bacterial counts of carcasses from broilers slaughtered after cooping (no feed withdrawal) and holding overnight. The average count of *Campylobacter* on carcasses slaughtered on the farm was \log_{10} 3.7 cfu/g ceca, as compared to an average of \log_{10} 7.1 cfu/g ceca for carcasses from broilers held overnight. In another field trial, these same researchers reported that transportation resulted in a 2.5 \log_{10} increase in *Campylobacter* contamination of ceca, but carcass contamination was not reported (Stern et al., 1995). Hoop and Ehram (1987) and Slader et al. (2002) indicated that *Campylobacter* could be isolated from transport coops even after cleaning and disinfecting and, thus, should be regarded as a potential source of *Campylobacter* contamination. The objective of the present study was to determine the effects of bird age at slaughter, feed withdrawal, and transportation on levels of *Campylobacter*, *Salmonella*, and *E. coli* on carcasses before and after immersion chilling.

MATERIALS AND METHODS

Birds

Campylobacter positive flocks were identified by analyses of cecal droppings using sterile BBL Culture Swabs² in commercial houses containing 28-d-old broilers. Six swabs were used to sample cecal droppings in each commercial house, and one house was evaluated on each of eight different farms. During sampling, one swab was placed into six separate cecal droppings. After sampling, swabs were placed into sterile tubes and the tubes were capped. Capped tubes were put into beakers on ice and transported to the laboratory for *Campylobacter* analysis. Birds were obtained only from houses containing *Campylobacter*-positive droppings.

One hundred eight, 30-d-old male broilers from each of two farms were caught, transported to the university research facility, and placed into pens with clean pine shavings. Nine birds from Farm 1 and 9 birds from Farm 2 were placed into each of 12 pens. Pens of broilers were assigned to one of two feed withdrawal (FW) treatments: 0 h FW (full fed) or 12 h FW. Birds were given access to feed (3,200 kcal ME/kg, 19% CP) ad libitum until FW was administered. One week prior to processing, birds were gavaged with nalidixic acid-resistant *Salmonella* (1×10^9 organisms in 1 mL/bird).

Processing

Two pens of broilers from each FW treatment were processed at 42, 49, and 56 d of age. Broilers in the 12-h FW treatment were held on litter with access to water but not feed for the first 4 h of the withdrawal period. These birds were then manually caught and crated in plastic coops (nine per coop). Four coops were placed as a single level in a pickup truck and driven for 1 h. After transportation, birds were held in coops for an additional 7 h before processing. Full-feed broilers (0 h FW) remained in pens with access to feed and water until 10 min before processing at which time they were caught, crated in plastic coops, and transported less than 0.2 km to the pilot plant facility. The processing plant was cleaned and sanitized with quaternary ammonium chloride³ before operation and between processing each treatment group. All birds were electrically stunned (two stage electrical stunner:⁴ 14 V, pulsed DC at approximately 500 Hz for 18 s, followed by 14 V, 60 Hz for 9 s), killed by hand using a conventional unilateral neck cut to sever the carotid artery and jugular vein, and bled for 120 s. During bleeding, cotton plugs were inserted into the cloaca, and birds were scalded for 2 min at 52 C in an air-agitated commercial scalders⁵ and picked for 36 s in a commercial in-line picker.⁶ After removing hocks, a manual opening cut was made on each carcass, and carcasses were mechanically eviscerated using an in-line automatic eviscerator.⁷ After a manual final wash, six carcasses (prechill) representing birds from each pen and FW treatment (three from Farm 1 and three from Farm 2 per pen) were subjected to a whole-carcass rinse (WCR). The remaining carcasses were tumble-chilled until deep breast muscle temperature was 4 C as measured by a digital thermometer inserted into a 22-ga hypodermic needle. Deep muscle temperature was monitored every 10 min.

Chillers were prepared by filling with ice and water to a final volume of 133 L. Approximately 40 mL of commercial bleach (6.15% sodium hypochlorite) was added to each chiller, and the total chlorine was measured using a CHEMetrics 2 SAM test kit.⁸ Prior to adding carcasses, total chlorine in the chillers was corrected to 20 ± 2 ppm by adding more water or sodium hypochlorite as noted by the test kit. Separate chillers were used for each FW treatment, and chilling times were recorded using a stopwatch. The tumble chillers were operated at approximately 2 rpm. After chilling, carcasses were hung in shackles and allowed to drip for 5 min. Six postchill carcasses representing birds from

²Becton Dickinson, Sparks, MD.

³AFCO Vigilquat, Alec C. Fergusson, Inc., Frazer, PA.

⁴Simmons model SF-7001, Simmons Engineering Co., Dallas, GA.

⁵Cantrell Model SS300CF, Cantrell Machine Co., Inc., Gainesville, GA.

⁶Cantrell Model CPF-60, Cantrell Machine Co., Inc., Gainesville, GA.

⁷Cantrell Model Mark 4, Cantrell Machine Co., Inc., Gainesville, GA.

⁸CHEMetrics Inc., Calverton, VA.

TABLE 1. Effect of age at slaughter on log₁₀ counts and number of carcasses testing positive (number positive/number tested) for coliforms, *Escherichia coli*, *Campylobacter*, and *Salmonella* and probabilities

Age (d)	Coliform	<i>E. coli</i>	<i>Campylobacter</i>	<i>Salmonella</i>
42	3.0 ^b (48/48)	2.2 ^b (48/48)	2.7 ^a (48/48)	1.2 ^a (12/48)
49	3.3 ^a (48/48)	2.5 ^{ab} (48/48)	2.2 ^b (40/48)	1.3 ^a (16/48)
56	3.4 ^a (48/48)	2.8 ^a (48/48)	2.0 ^b (43/48)	1.1 ^a (23/48)
<i>P</i>	0.033	0.006	0.0007	0.616

^{a,b}Means in the same column with no common superscripts differ significantly ($P < 0.05$).

each pen and FW treatment (three from Farm 1 and three from Farm 2 per pen) were subjected to a WCR.

Microbiological Analyses

Each carcass was placed into a clean plastic bag with 100 mL sterile PBS and shaken vigorously by hand in a one-foot arc for 60 s. Each carcass was aseptically removed from the bag, allowed to drain briefly into the bag, then discarded. Serial dilutions of the rinse were made in PBS, and *Campylobacter* was enumerated by plating in duplicate onto the surface of Campy-cefex agar (Stern et al., 1992). A 0.1-mL sample was spread on the surface of each plate with a sterile loop. Plates were incubated at 42 C for 36 h in a microaerophilic environment (5% O₂, 10% CO₂, and balance N₂). Colony-forming units characteristic of *Campylobacter* were counted. Each colony type identified as *Campylobacter* was confirmed for genus by examination of cellular morphology and motility on a wet mount under phase contrast microscopy. Each colony type was further identified for species *jejuni*, *coli*, or *lari* by a positive reaction on a latex agglutination test kit.⁹ Total aerobic bacterial populations were enumerated on plate count agar.¹⁰

A 0.1-mL sample from a serial dilution of the rinse diluent was plated in duplicate on the surface of the agar, spread, and incubated at 37 C for 18 to 24 h prior to counting the resulting colony-forming units. Coliform and *E. coli* counts were made by plating 1 mL from a serial dilution of the rinse diluent onto duplicate *E. coli* petrifilm plates.¹¹ Plates were incubated at 37 C for 18 to 24 h, and colony types characteristic of coliforms and *E. coli* were counted.

Statistical Analysis

Data were analyzed by the ANOVA option of the general linear models procedure of the SAS/STAT program using replicate, farm, age, FW, and site (before and after chilling) as the main effects (SAS Institute, 1999). All first-order interactions were tested for statistical significance ($P < 0.05$) using the residual error mean squares. Because there were no significant replicate, farm, or replicate-by-farm interac-

tion effects, the data were pooled by replicate and farm. Means were separated using the Duncan's multiple-range test ($P < 0.05$) option of SAS software using the appropriate error as described.

RESULTS AND DISCUSSION

Table 1 shows the effects of bird age at slaughter on log₁₀ counts of coliform, *E. coli*, *Campylobacter*, and *Salmonella* recovered from carcasses containing cloacal cotton plugs during evisceration. Coliform and *E. coli* counts increased with bird age, with the lowest counts for both microorganisms occurring on carcasses of 42-d-old broilers. The opposite was found with *Campylobacter* in which counts were 0.5 and 0.7 log₁₀ units higher on carcasses from 42-d-old birds as compared to counts on carcasses from broilers 49 and 56 d of age, respectively. Previous research regarding the relationship between bird age at slaughter and *Campylobacter* contamination has reported conflicting results. Smitherman et al. (1984) conducted a survey of *Campylobacter jejuni* by analyzing cloacal swabs and fecal droppings on four different commercial farms. For three out of four farms, these authors noted that the probability of *Campylobacter* infection increased with bird age. Pokamunski et al. (1986) reported that the number of ceca testing positive for *Campylobacter* was slightly higher for 8-wk-old broilers (85/120) as compared to ceca testing positive for 4-wk-old broilers (72/120). Jacobs-Reitsma et al. (1994) conducted a year-long study on *Campylobacter* and *Salmonella* in 187 broiler flocks and found that age at slaughter had no significant influence on the presence of *Campylobacter* or *Salmonella*. These authors did, however, report a positive correlation between *Campylobacter* and *Salmonella* colonization within flocks.

In the present study, *Salmonella* counts did not vary with bird age (Table 1). This result is likely related to the age at which live birds were gavaged with *Salmonella*. Birds thought to be naturally harboring *Campylobacter* (based on cecal dropping analyses) were inoculated with *Salmonella* 1 wk before processing (ages 35, 42, and 49 d). The WCR indicated that intestinal colonization with *Salmonella* was limited (*Salmonella* log₁₀ counts < 1.5). This result is possibly because the microflora of these birds was already established before they were gavaged. Previous research has shown that it is difficult to achieve intestinal colonization with bacteria administered to older broilers (Bryan and Doyle, 1995).

⁹Integrated Diagnostics Inc., Baltimore, MD.

¹⁰Becton Dickinson, Sparks, MD.

¹¹3M Health Care, St. Paul, MN.

TABLE 2. Effect of feed withdrawal (FW) on log₁₀ counts and number of carcasses testing positive (number positive/number tested) for coliforms, *Escherichia coli*, *Campylobacter*, and *Salmonella* and probabilities

FW (h)	Coliform	<i>E. coli</i>	<i>Campylobacter</i>	<i>Salmonella</i>
0	3.2 (72/72)	2.4 (72/72)	2.1 ^b (65/72)	1.1 (24/72)
12	3.3 (72/72)	2.6 (72/72)	2.5 ^a (66/72)	1.3 (26/72)
<i>P</i>	0.514	0.106	0.0403	0.449

^{a,b}Means in the same column with no common superscripts differ significantly ($P < 0.05$).

The FW was observed to have a significant affect on carcass *Campylobacter* counts but had no effect on carcass coliform, *E. coli*, or *Salmonella* counts (Table 2). This finding may be related to the limited intestinal leakage during evisceration provided by the use of cotton plugs in the cloacae of carcasses. Previous research has shown that carcasses may become contaminated with intestinal contents that leak from the crop or cloaca during evisceration (Wabeck, 1972; Bilgili, 1988; Papa and Dickens, 1989; May et al., 1990; Papa, 1991; Thayer and Walsh, 1993; Northcutt et al., 1997; Buhr et al., 1998). Additionally, research has shown that there is a relationship between broiler feed withdrawal and fecal moisture (Wabeck, 1972). Wabeck (1972) reported that broilers held without feed for 12 or 24 h had increased amounts of moisture in their feces compared with broilers held without feed for 8 to 10 h. In the present study, attention was focused on exterior contamination of live broilers.

Log₁₀ *Campylobacter* was observed to increase only slightly (0.4 U) when feed was withdrawn for 12 h before slaughter (Table 2), which included the 1-h transportation and 7-h holding time in coops. The effects of FW on *Campylobacter* appear to be primarily associated with older broilers (age 56) before chilling. For the 56-d-old broilers, carcasses from birds held without feed (12 h) had higher log₁₀ counts (average count log₁₀ 2.8) than carcasses from birds processed full fed (average count log₁₀ 2.2). Although these differences were statistically significant, a 0.4 to 0.6 log₁₀ difference would most likely be insignificant to a commercial operation. The results of the present study are, however, in agreement with those previously reported by Stern et al. (1995) for carcass *Campylobacter* levels before and after transportation (including holding without feed). After chilling, length of FW had no effect on any of the bacteria counts.

Table 3 shows the effect of sampling before and after chilling on log₁₀ counts and number of carcasses testing positive for coliforms, *E. coli*, *Campylobacter*, and *Salmonella*. Immersion chilling of carcasses resulted in a log₁₀ reduction of 1.3, 1.4, 1.3, and 0.5 for coliforms, *E. coli*, *Campylobacter*, and *Salmonella*, respectively (Table 3). Previous research has reported that although bacteria counts on broiler carcasses decrease as carcasses move through the processing plant, the number of carcasses testing positive for *Salmonella* increases after immersion chilling (Lillard, 1986). Similar findings have been reported by Jones et al. (1991) for *Campylobacter*, indicating that cross contamination with *Salmonella* and *Campylobacter* may increase during processing while actual counts may decrease.

In the present study, prototype immersion chillers were used with capacities of 133 L. This volume makes it difficult to compare the results of the present study with those observed commercially, in which thousands of carcasses are chilled simultaneously and fresh water input is 1.9 L/bird. In the present study, an initial concentration of approximately 20 ppm total chlorine was added to the immersion chillers, whereas the final concentration of free chlorine was ≤0.2 ppm after chilling. Izat et al. (1988) indicated that carcass *Campylobacter* levels are reduced in processing plants wherever water is used, and they reported a 1 to 2.2 log₁₀ *Campylobacter* reduction for carcasses after immersion chilling. Similar findings have been reported by Oosterom et al. (1983) and Jones et al. (1991).

Previous research has suggested that the majority of the *Campylobacter* contamination during processing (broiler carcasses, processing lines, workers' hands, and finished products) originates from intestinal leakage, cuts, or tears (Oosterom et al., 1983; Genigeorgis et al., 1986; Berndtson et al., 1992). Thus, a likely conclusion would be that coliforms, *E. coli*, *Campylobacter*, and *Salmonella* were trans-

TABLE 3. Effect of sampling before or after chilling (site) on log₁₀ counts and number of carcasses testing positive (number positive/number tested) for coliforms, *Escherichia coli*, *Campylobacter*, and *Salmonella* and probabilities (*P*)

Site	Coliform	<i>E. coli</i>	<i>Campylobacter</i>	<i>Salmonella</i>
Before chilling	3.9 ^a (72/72)	3.2 ^a (72/72)	2.9 ^a (71/72)	1.3 ^a (40/72)
After chilling	2.6 ^b (72/72)	1.8 ^b (72/72)	1.6 ^b (60/72)	0.8 ^b (11/72)
<i>P</i>	0.0001	0.0001	0.0001	0.009

^{a,b}Means in the same column with no common superscripts differ significantly ($P < 0.05$).

ferred to carcasses from the birds' intestines during processing. However in this study, a cotton plug was inserted in the cloaca of each carcass during bleeding to prevent intestinal leakage. Cotton plugs in the cloaca have been shown to significantly reduce the transfer of intestinal bacteria to the exterior of the carcass (Stern et al., 1995; Musgrove et al., 1997). Musgrove et al. (1997) reported finding nearly eight times more colony-forming units of *Campylobacter* spp. on control (nonplugged) versus plugged carcasses. Based on their findings, it may be concluded that the majority of the bacteria recovered in the present study were from the birds' exteriors. Furthermore, these data revealed that FW (including transportation) may have increased prechill carcass counts for *E. coli* and *Campylobacter*, particularly in older broilers, but it had no effect on postchill counts when chlorine was used.

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